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Increased carbonyl modification by lipids and carbohydrates in diabetic nephropathy

TOSHIO MIYATA, SATOSHI SUGIYAMA, DAISUKE SUZUKI, REIKO INAGI, and KIYOSHI KUROKAWA

Molecular and Cellular Nephrology, Institute of Medical Sciences and Department of Internal Medicine, Tokai University School of Medicine, Isehara, and Department of Nephrology, Chukyo Hospital, Nagoya, Japan

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Background. In diabetic nephropathy (DN), possible mediators of untoward effects of hyperglycemia include the advanced glycation end products (AGEs). Indeed, an AGE, carboxymethyllysine (CML), accumulates in expanded mesangial matrix and nodular lesions. An advanced lipoxidation end product (ALE), malondialdehyde-lysine (MDA-lysine), generated on proteins during lipid peroxidation also accumulates in these lesions. As both ALEs and AGEs are formed by carbonyl amine chemistry between protein and carbonyl compounds derived from autooxidation of lipids and carbohydrates, their colocalization suggests an increased carbonyl modification of proteins.

Methods. To address this hypothesis, human diabetic renal tissues were examined to characterize carbonyl modification of proteins by lipids and carbohydrates: (a) ALEs, MDA-lysine and 4-hydroxynonenal (HNE) protein adduct, derived from lipids, and (b) AGEs, pentosidine and CML, derived from carbohydrates. Furthermore, to elucidate the biological effect of carbonyl modification on primary cultured human and rat mesangial cells, the intracellular protein phosphorylation was examined in the presence of various kinds of carbonyl compounds.

Results. The ALE and AGE adducts examined were identified in expanded mesangial matrix and nodular lesions. The exposure of cultured mesangial cells to carbonyl compounds resulted in phosphorylation of tyrosine residues of a number of intracellular proteins.

Conclusions. These data suggest a broad derangement in nonenzymatic biochemistry involving both lipids and carbohydrates exists in diabetic glomerular lesions ("carbonyl stress").

Diabetic nephropathy (DN) has become one of the main causes of end-stage renal disease. The metabolic events responsible for its development are not understood well. Possible mediators of untoward effects of hyperglycemia include the advanced glycation end products (AGEs) generated by nonenzymatic glycation and oxidation reactions.

Key words: diabetes, advanced glycation end products, advanced lipoxidation end product, reactive carbonyl compound, oxidative protein damage.

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Advanced glycation end products accumulate in plasma and tissue proteins of diabetic patients [1, 2], and their accumulation is correlated with the severity of diabetic complications [2, 3]. AGEs comprise a variety of molecular structures, such as N^ε-(carboxymethyl)lysine (CML), pentosidine, and pyrraline, which are characterized by different formation mechanisms. Glycooxidation products, a subclass of AGEs that requires both glycation and oxidation for their formation, such as CML and pentosidine, accumulate in expanded mesangial matrix and nodular lesions in DN [4]. By contrast, pyrraline, another AGE structure in which the deposition is rather independent from oxidative stress, was not found within diabetic glomeruli. Interestingly, the distribution of malondialdehyde (MDA)-lysine, an advanced lipoxidation end product (ALE), colocalized with the CML and pentosidine distribution. We therefore hypothesized that the localization of CML, pentosidine, and MDA-lysine, in which the formation is closely related to oxidative process, is independent evidence for a local oxidative stress in diabetic glomerular lesions [4].

INCREASED CARBONYL MODIFICATION OF GLOMERULAR PROTEINS BY LIPIDS AND CARBOHYDRATES: "CARBONYL STRESS"

Under oxidative stress, proteins may be modified either directly by reactive oxygen species with the eventual formation of oxidized amino acids or indirectly by reactive carbonyl compounds formed by autooxidation of carbohydrates and lipids. Both AGEs and MDA-lysine are indeed products formed by carbonyl amine chemistry between protein amino group and carbonyl compounds derived from autooxidation of carbohydrates and lipids (see [5] for a review of the structures of carbonyl compounds and their final protein adducts). Autooxidation of carbohydrates yields reactive carbonyl compounds, precursors of AGEs, such as glyoxal, arabinose, and glycolaldehyde, as well as dehydroascorbate formed on oxidation of ascorbate [5]. Lipid peroxidation yields other

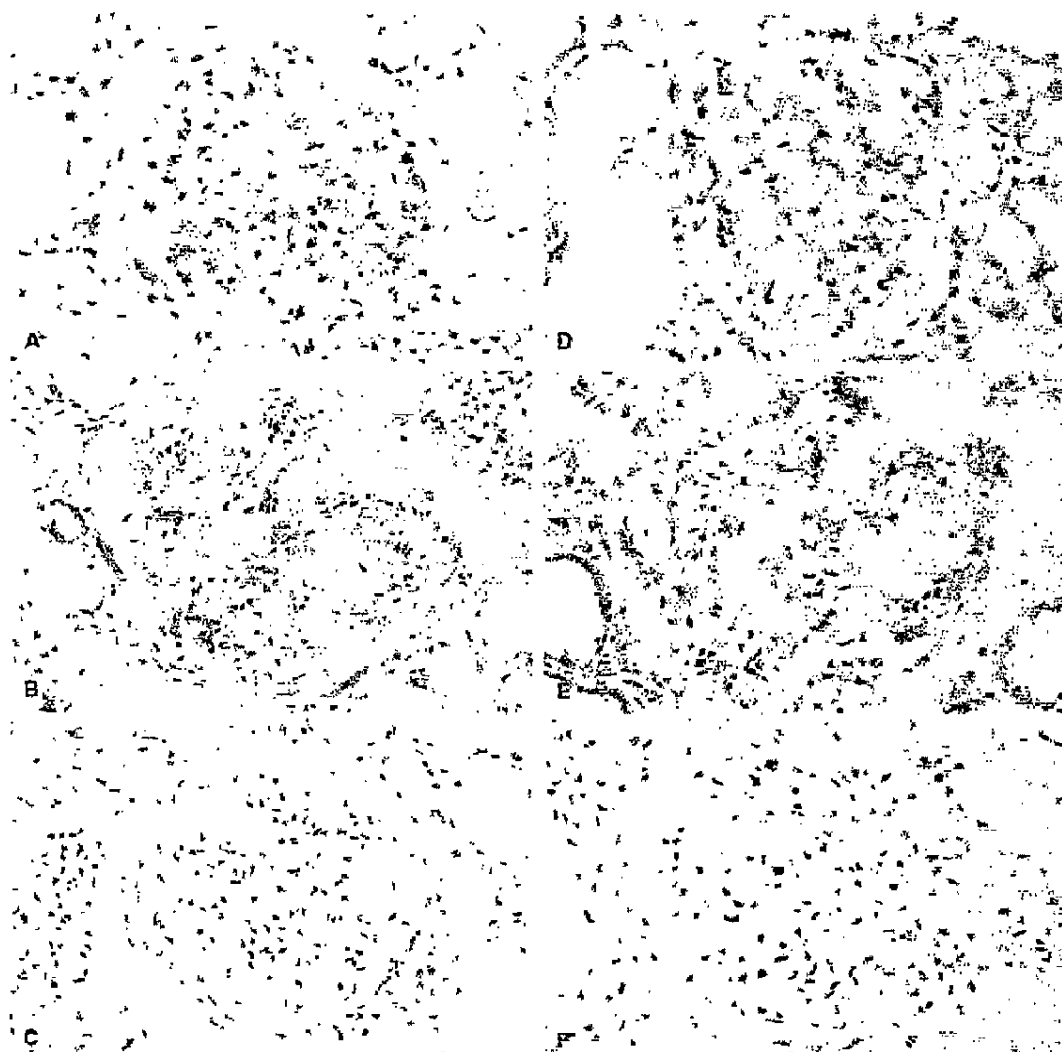


Fig. 1. Immunohistochemical detection of advanced glycation end product (AGE; A-C) and advanced lipoxidation end product (ALE; D-F) in diabetic glomerular lesions. Both carboxymethyllysine (CML; AGE) and malondialdehyde (MDA)-lysine (ALE) were identified in the expanded mesangial area (A and D) and in nodular lesions (B and E) of diabetic nephropathy, whereas their immunostainings were faint in nondiabetic normal glomeruli (C and F). Anti-AGE mouse monoclonal IgG (6D12), the major epitope structure of which was identified as CML, and anti-MDA-lysine mouse monoclonal IgG (kindly provided from Dr. Joseph L. Witztum) were used for immunohistochemistry. The characterization of the antibodies was described previously [4]. The specificity of immunostaining was confirmed by competition experiments with either anti-CML or anti-MDA-lysine antibody preincubated with an excess of CML-bovine serum albumin or MDA-bovine serum albumin, respectively (original magnification $\times 200$).

reactive carbonyl compounds, such as MDA and 4-hydroxynonenal (HNE). These carbonyl compounds form ALEs on proteins, such as MDA-lysine and HNE protein adduct [5].

Therefore, the colocalization of both AGEs and ALEs suggests not only an increased local oxidative stress, but also an increased carbonyl modification of proteins in diabetic glomerular lesions. To address this hypothesis,

human diabetic renal tissues were examined with specific antibodies to characterize carbonyl modification of proteins by autooxidation products of carbohydrates and lipids in (a) AGEs (CML and pentosidine), derived mainly from carbohydrates, and (b) ALEs (MDA-lysine and HNE protein adduct), derived mainly from lipids. All of the protein adducts formed by carbonyl amine chemistry were identified immunohistochemically in the character-

istic DN lesions, that is, the expanded mesangial matrix and nodular lesions (exemplified for CML and MDA-lysine in Fig. 1 A, B, D and E, respectively). On the other hand, their immunostainings were faint in nondiabetic normal glomeruli (Fig. 1, C, F). The antibodies used recognize distinct structures and do not cross-react with the other structures. Therefore, the distributions of all the protein adducts formed by carbonyl amine chemistry in DN are independent measures for an increased carbonyl modification of proteins ("carbonyl stress").

Thus, the metabolic and chemical imbalances characteristic in diabetic glomerular lesions might be described more appropriately as carbonyl stress rather than advanced glycation or oxidative stress alone. Carbonyl stress implicates a much broader derangement in nonenzymatic biochemistry that involves both lipids and carbohydrates.

PATHOLOGICAL SIGNIFICANCE OF CARBONYL STRESS IN THE DEVELOPMENT OF DIABETIC GLOMERULAR LESIONS

The presence of carbonyl stress suggests its role in the development of diabetic glomerular lesions. Indeed, the AGE- or ALE-modified proteins stimulate a variety of cellular responses, including glomerular mesangial cells [6-8]. Furthermore, carbonyl stress not only induces inflammatory responses, but may also have direct biological effects on cells. As most of the carbonyl compounds have more than two carbonyl groups within the moiety, they might covalently cross-link matrix tissue proteins and alter their structure and function, or they might cross-link cell surface proteins and stimulate cellular responses. We recently demonstrated the increased intracellular protein-tyrosine phosphorylation in murine thymocytes and fibroblasts by interaction with carbonyl compounds, such as glyoxal and MDA [9]. This increased protein-tyrosine phosphorylation may be attributed to the cross-linking of cell surface proteins and the subsequent activation of some protein tyrosine kinase, such as c-Src [9]. The protein-tyrosine phosphorylation in murine thymocytes and fibroblasts by interaction with carbonyl compounds is inhibited by pretreatment with 2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide (OPB-9195), an inhibitor of carbonyl-amine reactions [5, 9]. A number of intracellular proteins are also phosphorylated on tyrosine residues in primary cultured hu-

man and rat mesangial cells exposed to carbonyl compounds (abstract; Yasuda et al, *J Am Soc Nephrol* 9:645A, 1998).

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Reprint requests to Toshio Miyata, M.D., Ph.D., Molecular and Cellular Nephrology, Institute of Medical Sciences and Department of Internal Medicine, Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa 259-11, Japan.

APPENDIX

Abbreviations used in this article are: AGE, advanced glycation end product; ALE, advanced lipoxidation end product; CML, N^ε-(carboxymethyl)lysine; DN, diabetic nephropathy; HNE, 4-hydroxynonenal; MDA, malondialdehyde.

REFERENCES

1. DYER DG, DUNN JA, THORPE SR, BAILIE KE, LYONS TJ, MCCANCE DR, BAYNES JW: Accumulation of Maillard reaction products in skin collagen in diabetes and aging. *J Clin Invest* 91:2463-2469, 1993
2. SUGIYAMA S, MIYATA T, UEDA Y, TANAKA H, MAEDA K, KAWASHIMA S, VAN YPERSELE DE STRIHOUC C, KUROKAWA K: Plasma level of pentosidine, an advanced glycation end product, in diabetic patients. *J Am Soc Nephrol* 9:1681-1688, 1998
3. MCCANCE DR, DYER DG, DUNN JA, BAILIE KE, THORPE SR, BAYNES JW, LYONS TJ: Maillard reaction products and their relation to complications in insulin-dependent diabetes mellitus. *J Clin Invest* 91:2470-2478, 1993
4. HORIE K, MIYATA T, MAEDA K, MIYATA S, SUGIYAMA S, SAKAI H, VAN YPERSELE DE STRIHOUC C, MONNIER VM, WITZTUM JL, KUROKAWA K: Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions. *J Clin Invest* 100:2995-3004, 1997
5. MIYATA T, VAN YPERSELE DE STRIHOUC C, KUROKAWA K, BAYNES JW: Alterations in nonenzymatic biochemistry in uremia: Origin and significance of "carbonyl stress" in long-term uremic complications. *Kidney Int* 55:389-399, 1999
6. SKOLNIK EY, YANG Z, MAKITA Z, RADOFF S, KIRSTEIN M, VLASSARA H: Human and rat mesangial cell receptors for glucose-modified proteins: Potential role in kidney tissue remodeling and diabetic nephropathy. *J Exp Med* 174:931-939, 1991
7. MIYATA T, INAGI R, IIDA Y, SATO M, YAMADA N, ODA O, MAEDA K, SEO H: Involvement of β_2 -microglobulin modified with advanced glycation end products in the pathogenesis of hemodialysis-associated amyloidosis: Induction of human monocyte chemotaxis and macrophage secretion of tumor necrosis factor- α and interleukin-1. *J Clin Invest* 93:521-528, 1994
8. MIYATA T, HORI O, ZHANG J, YAN SD, FERRAN L, IIDA Y, SCHMIDT AM: The receptor for advanced glycation end products (RAGE) is a central mediator of the interaction of AGE- β_2 -microglobulin with human mononuclear phagocytes via an oxidant-sensitive pathway. *J Clin Invest* 98:1088-1094, 1996
9. AKHAND AA, KATO M, SUZUKI H, HAMAGUCHI M, MIYATA T, KUROKAWA K, NAKASUMA I: Carbonyl stress modifies cell surface proteins for activating protein-tyrosine kinase p60^{src}. *J Cell Biochem* 72:1-7, 1999